EFFECTS OF SALINITY ON NUTRITIONAL REQUIREMENTS OF *ARTEMIA SALINA*

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Until recently, most of the work being done on the problem of metabolism in Crustacea centered around hormonal control (McWhinnie and Corkill, 1964). The importance of nutritional state has been emphasized (McWhinnie and Kirchenberg, 1962) but very little is known about the metabolic function of nutrients. The development of an artificial medium for *Artemia* salinia by Provasoli and d'Agostino (1969) offers a good opportunity to study this problem.

Information is lacking on how salinity affects the nutritional requirements of *Artemia* (d'Agostino and Provasoli, 1968). Besides gathering some general metabolic data, a further object of this investigation was to study the morphogenetic effect of adenosine monophosphate (AMP) deficiency. AMP deficiency induces a supernumerary gonopode morphogenesis and reduces abdominal length (Hernandorena, 1970, 1972a). It is well known that *Artemia* reared at different salinities shows certain modification in form. In particular, with an increasing salinity of the medium the abdomen grows relatively longer and narrower (Gilchrist, 1960).

Extremes of salinity nonspecifically reduce incorporation of radiocarbon from $2-^{14}C$ sodium acetate into the intermediates of tricarboxylic acid cycle of *Artemia* (Huggins, 1969). Succinodehydrogenase activity varies with oxygen consumption in *Artemia* (Packard and Taylor, 1968) and oxygen consumption decreases with increasing salinity (Eliassen, 1952; Dutrieu, 1960; Engel and Angelovic, 1968).

So it might be expected that salinity modifies AMP metabolism and consequently morphogenesis.

MATERIALS AND METHODS

The method developed by Provasoli and d'Agostino (1969) for the Utah strain axenic cultivation was used. The medium is composed of: (1) a liquid phase containing mineral salts, six amino acids, eight vitamins, two sugars and a mixture of nucleic derivatives from which guanylic acid has been omitted and has the following composition: adenylic acid, 60 mg%; uridylic acid, 10 mg%; cytidylic acid, 10 mg%; and thymidine, 5 mg%; and

(2) a fine particulate phase composed of egg albumin, rice starch and cholesterol. Duplicated experiments each using 2×10 ml of medium were used for all the treatments. The criteria used to evaluate the results were the growth index established according to Provasoli and d'Agostino (1969) numerical method and the survival percentage (Hernandorena, 1972a, 1972b and 1972c).

RESULTS

The optimal starch and albumin concentrations in medium "100" by Provasoli and d'Agostino (1969) have been checked in our experimental conditions.
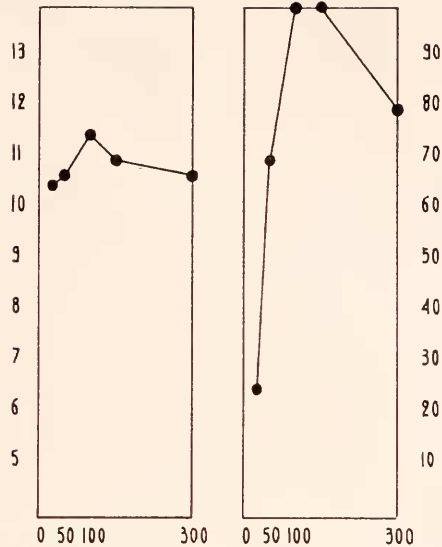
FIGURE 1. Effect of starch concentration; both abscissae represent starch in mg%; albumin constant at 20 mg%. In all the figures, growth is represented in the left-hand, and survival in the right-hand graph, the left-hand ordinate being the growth index for the 14th day of development, and the right-hand ordinate being the survival percentage for index 10 (end of larval life).

With albumin constant at 20 mg%, growth index and survival percentage increased with starch concentration up to a 100 mg% level (Fig. 1), and declined at higher concentrations. With starch constant at 100 mg%, growth index increased
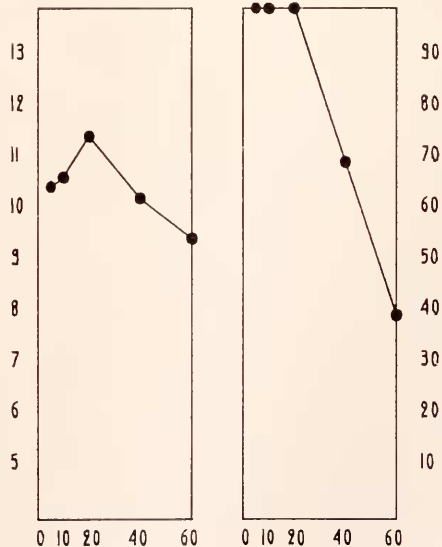

FIGURE 2. Effect of albumin concentration, both abscissae albumin in mg%; starch constant at 100 mg%; ordinates as in Figure 1.
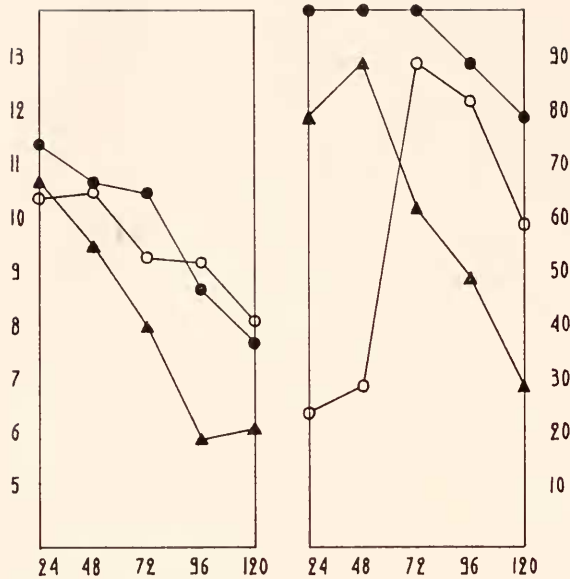
FIGURE 3. Effect of salinity on starch requirement; both abscissae NaCl in g‰; starch: open circles, 25 mg%; closed circles, 100 mg%; closed triangles, 300 mg%; ordinates as in Figure 1.

with albumin concentration up to a 20 mg% level (Fig. 2). These results are similar to those found by Provasoli and d'Agostino although albumin deficiency is partially relieved by supplementary amino acids.

Varying salinity affects the starch and albumin requirements. Starch deficiency is less detrimental and a starch concentration above optimum is more detrimental with increasing salinity (Fig. 3). Albumin deficiency is more detrimental and above optimal albumin concentration less detrimental with increasing salinity (Fig. 4).

According to Provasoli and d'Agostino (1969) as the level of albumin increases more starch is needed for optimal growth. Starch is replaceable by lecithin (Fig. 5). With starch constant at 100 mg%, lecithin is tolerated up to a 2 mg% level (Hernandorena, 1972b). Lecithin also relieves the detrimental effect of excess albumin (Fig. 6); thus confirming the need for a ratio energetic nutrient: albumin.

The optimal starch: albumin ratio stands somewhere between 5:1 and 3.3:1 (Provasoli and d'Agostino, 1969). This relationship holds true at 24‰ but not at 120‰: in a starch deficient medium (50 mg%) more albumin is needed for optimal growth (Fig. 7).

With starch constant at 100 mg%, lecithin addition (2 mg%) increases growth index at 24‰ salinity but becomes inhibitory with increasing salinity (Fig. 8) thus confirming the reduction of energetic nutrient requirement with increasing salinity.

AMP requirement depends on albumin concentration. Suboptimal AMP (20 mg%) becomes more detrimental as the albumin concentration increases (Fig. 9). In an albumin deficient medium (5 mg%) the supernumerary gonopode morpho-
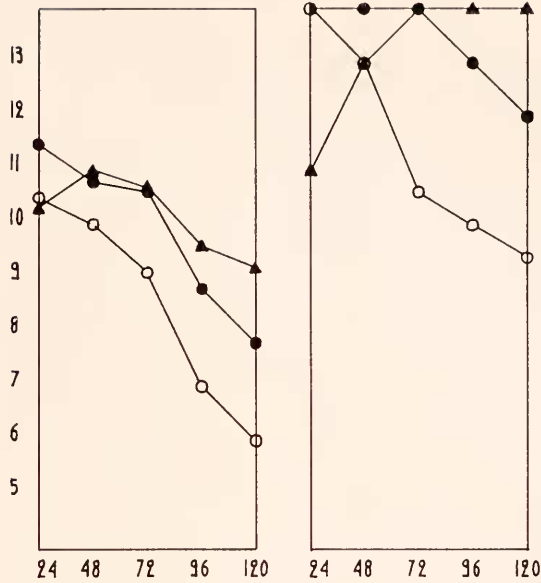
FIGURE 4. Effect of salinity on albumin requirement; both abscissae NaCl in g%; albumin: open circles, 5 mg%; closed circles, 20 mg%; closed triangles, 40 mg%; ordinates as in Figure 1.
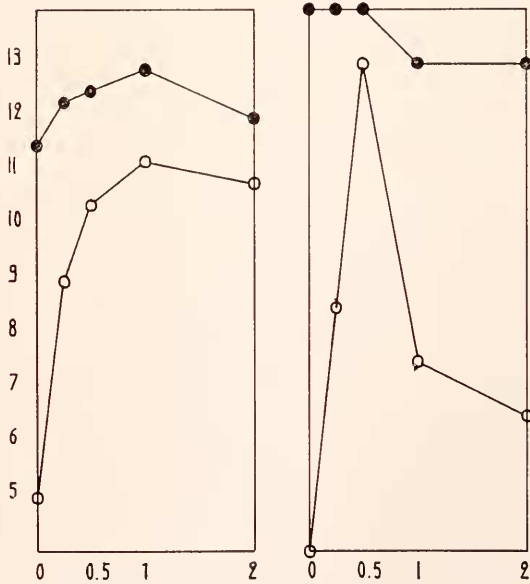

FIGURE 5. Effect of lecithin; both abscissae lecithin in mg%; open circles, no starch; closed circles, starch 100 mg%; ordinates growth and survival, see Figure 1.
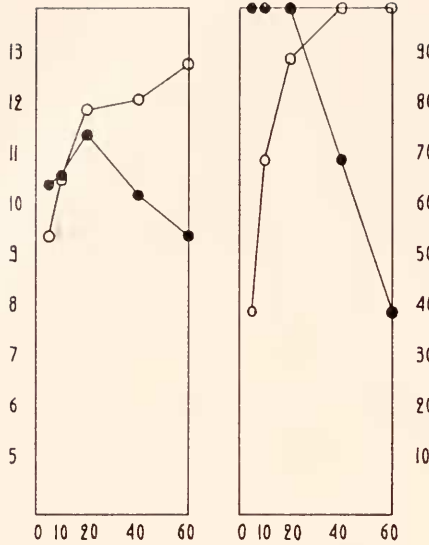
FIGURE 6.   Effect of albumin concentration on lecithin requirement; both abscissae albumin in mg%; closed circles, no addition; open circles, lecithin 2 mg%; ordinates growth and survival, see Figure 1.

genesis is no longer induced by AMP deficiency.   More AMP is needed for optimal growth in a medium containing an above optimal (60 mg%) albumin concentration (Fig. 10).   In a starch deficient medium (up to 10 mg%) growth index and
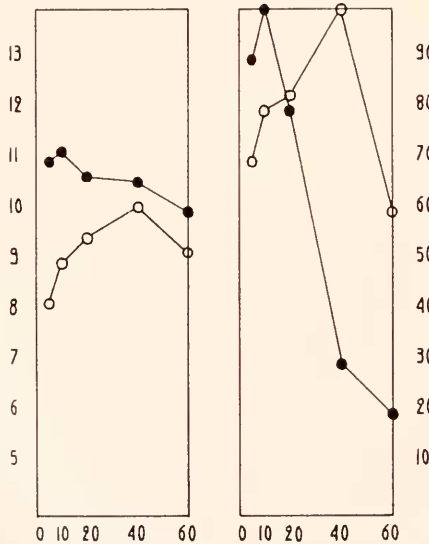

FIGURE 7.   Effect of salinity on albumin requirement; both abscissae albumin in mg%; starch constant at 50 mg%; salinity: closed circles, 24%; open circles, 120%; ordinates growth and survival, see Figure 1.
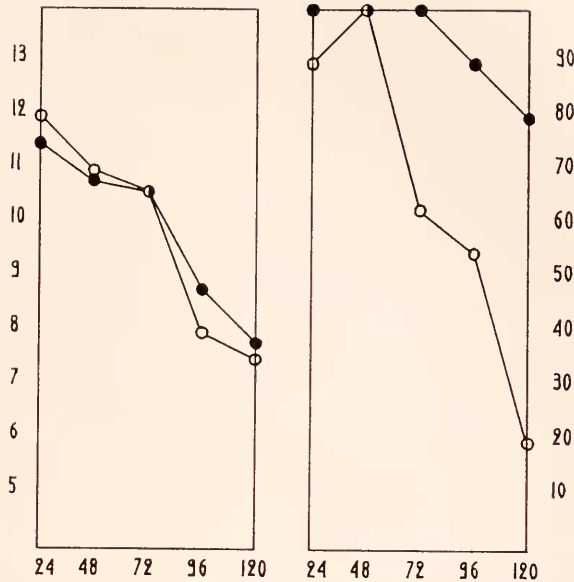
FIGURE 8. Effect of salinity on lecithin requirement; both abscissae NaCl in g‰; starch constant at 100 mg%; albumin constant at 20 mg%; AMP constant at 60 mg%; closed circles, no addition; lecithin: open circles, 2 mg%; ordinates growth and survival, see Figure 1.

survival percentage do not increase with AMP concentration. With starch constant at 100 mg%, growth index and survival percentage increase with AMP concentration up to a 140 mg% level (Fig. 11). With increasing salinity, less AMP is
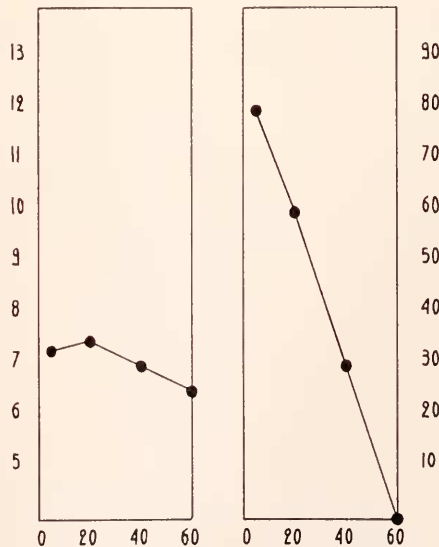

FIGURE 9. Effect of albumin concentration on AMP requirement; both abscissae albumin in mg%; AMP constant at 20 mg%; ordinates growth and survival, see Figure 1.
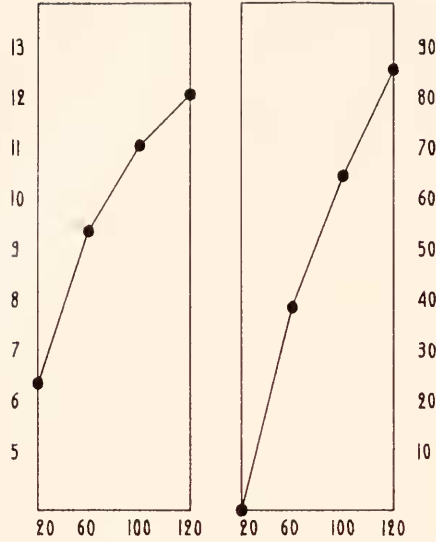
FIGURE 10. Effect of high albumin concentration on AMP requirement; both abscissae AMP in mg%; albumin constant at 60 mg%; ordinates growth and survival, see Figure 1.

needed for optimal growth. Growth index and survival percentage increase with AMP concentration up to a 100 mg% level at 72‰ and to a 60 mg% level at 120‰ salinity.
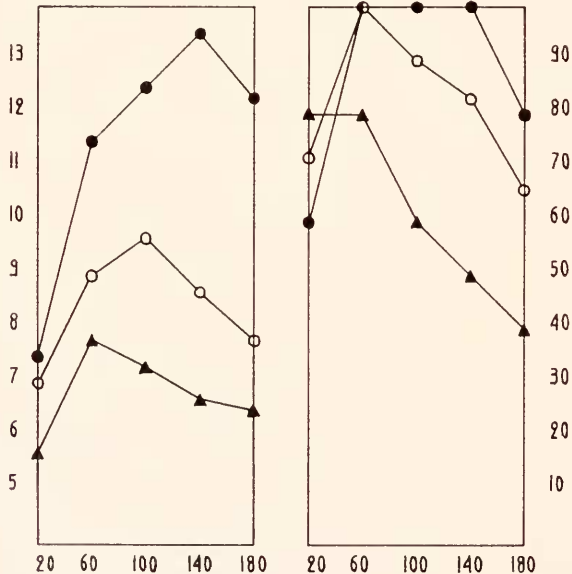

FIGURE 11. Effect of salinity on AMP requirement; both abscissae AMP in mg%; starch constant at 100 mg%; albumin constant at 20 mg%; salinity; closed circles, 24‰; open circles, 72‰; closed triangles, 120‰; ordinates growth and survival, see Figure 1.
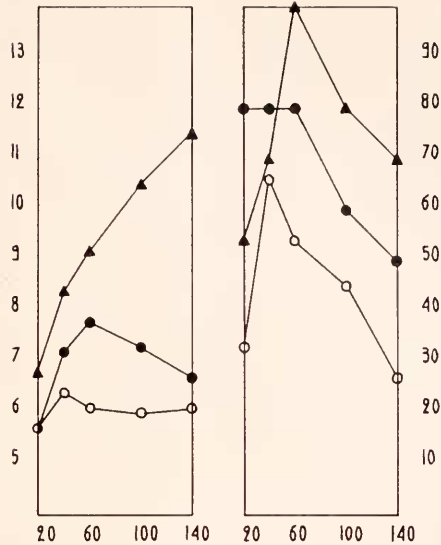
FIGURE 12. Albumin and AMP requirements at high salinity (120‰); both abscissae AMP in mg%; albumin: open circles, 5 mg%; closed circles, 20 mg%; closed triangles, 40 mg%; ordinates growth and survival, see Figure 1.

At 120‰ salinity, albumin deficiency is detrimental whatever AMP concentration. Growth index increases with AMP concentration only for above optimal (40 mg%) albumin concentration (Fig. 12). In an AMP deficient medium (20 mg%) the supernumerary gonopode morphogenesis is induced only with an albumin excess (40 mg%). These results confirm the higher albumin requirement and lower AMP requirement with increasing salinity.

DISCUSSION

The problem of energy sources in Crustacea was studied by Renaud (1949) and the results of her work led Vonk (1960) to conclude that Crustacea metabolism is mainly centered around glycogen and fatty acids. But according to Huggins and Munday (1968) the relative significance of carbohydrate in meeting the basic energy requirement still requires further investigation. The large amount of free amino acids in Crustacea and the variability of this amount under conditions leading to an energetic adjustment may be considered as indicating the use of amino acids in the process of energy production (Gilles, 1970). In *Artemia* medium the energy requirement is met by starch or lecithin at 24‰ salinity. More energetic nutrient and more AMP were needed for optimal growth as the albumin level increased. The *Artemia* purine nucleotide requirement is specific and absolute. With a given albumin concentration, growth rate and abdominal length increase with AMP concentration (Hernandorena, 1972b). In *Drosophila* developmental rate and ultimate size depend on the protein concentration of the diet (Sang, 1959). Dietary RNA requirement depends on protein concentration (Geer, 1963) and RNA requirement reflects essentially an AMP requirement (Sang, 1959). But dietary AMP requirement is not an absolute requirement in all

Drosophila strains (Hinton, Ellis and Noyes, 1951). In *Artemia* the quantitative nature of AMP requirement is relative and depends on the albumin concentration of the diet. At 24‰ salinity, the optimal growth rate is achieved with a proper energetic nutrient + AMP: albumin ratio. The protein concentration is highest during exponential growth indicating that almost all growth is due to new protein synthesis (Dagg, 1969); amino acid incorporation is used as a criterion for protein synthesis. Oxidative metabolism is essential for amino acid incorporation into tissue protein. Both glycolytic and Krebs cycle intermediates as well as ATP stimulate the incorporation of glycine into proteins of *Bombyx* (Chefurka, 1965). Since the morphogenetic effect of AMP deficiency depends on albumin concentration and salinity, the AMP morphogenetic effect should be looked for at the protein synthesis level.

With increasing salinity, starch or lecithin requirements are reduced. The incorporation of radiocarbon from 2-^{14}C sodium acetate into the intermediates of the Krebs cycle is reduced by salinity (Huggins, 1969), and as a consequence, it seems that the energy requirement is no longer met by starch or lecithin.

With increasing salinity, the albumin requirement is increased. The results of Emerson (1967) on the free amino acid level of *Artemia* nauplii incubated at different salinities led Huggins (1969) to suggest the particular importance of proline biosynthesis in adaptation to salinity by *Artemia*. Evidence has been presented supporting the use of proline as an energy reserve in *Aedes aegypti* (Thayer, 1972). Utilization of dietary amino acids in lipid synthesis by aseptically reared *Musca domestica* has been demonstrated, leucine and glutamate being the best lipid precursors (Kon and Monroe, 1971). The role of individual amino acids in adaptation to salinity and energy production by *Artemia* is under study.

Another point requires further investigation. The results of Reeve (1963) led Provasoli and d'Agostino (1969) to conclude that in medium "100" *Artemia* is ingesting particles at a maximum constant rate. *Artemia* ingested liquids but apparently to a very limited extent. Direct uptake of phosphate ion from the medium has been effectively demonstrated (Kobayashi, Saita and Tomiyama 1972). According to Croghan (1958a, 1958b) *Artemia* regulates the osmotic pressure and ionic balance of its hemolymph by taking up NaCl and water from the gut lumen and excreting NaCl through the branchiae. Osmotrophy is limited at 24‰ but may increase at 120‰ since the drinking rate of the eel has been shown to vary in relation to external salinity (Maetz and Skadhange, 1968).

SUMMARY

1. At 24‰ salinity the energy requirement of *Artemia* is met by starch or lecithin.

2. The need for a starch: albumin ratio put forward by Provasoli and d'Agostino (1969) has been extended to a need for an energetic nutrient + AMP: albumin ratio. With increasing salinity, the eneregtic nutrient requirement decreases and the albumin requirement increases.

3. AMP deficiency induces a supernumerary gonopode morphogenesis. The quantitative nature of AMP deficiency inducing the morphogenetic action depends on albumin concentration and salinity. The AMP morphogenetic effect should be looked for at the protein synthesis level.

4. The possible significance of salinity induced modifications in energetic nutrient and albumin requirements is discussed.

LITERATURE CITED

CHEFURKA, W., 1965. Metabolism of nitrogenous and lipid compounds. Pages 669–768 in M. Rockstein, Ed., *The Physiology of Insecta,* vol. II. Academic Press.

CROGHAN, P. C., 1958a. The mechanism of osmotic regulation in *Artemia salina* (L.) The physiology of the branchiae. *J. Exp. Biol.,* **35**: 234–242.

CROGHAN, P. C., 1958b. The mechanism of osmotic regulation in *Artemia salina* (L.) The physiology of the gut. *J. Exp. Biol.,* **35**: 243–249.

DAGG, M. J., 1969. Relationship between growth rate and RNA, DNA protein and dry weight in *Artemia salina* and *Euchaeta japonica. M.S. thesis, University of Victoria,* Victoria, B.C.

D'AGOSTINO, A., AND L. PROVASOLI, 1968. Effects of salinity and nutrients on mono- and diaxenic cultures of two strains of *Artemia salina. Biol. Bull.,* **134**: 1–14.

DUTRIEU, J., 1960. Observations biochimiques et physiologiques sur le développement d'*Artemia salina* (L.). *Arch. Zool. Exp. Gen.,* **99**: 1–133.

ELIASSEN, E., 1952. Energy metabolism of *Artemia salina* in relation to body size seasonal rhythms and different salinities. *Universitet Bergen Arbok, Naturvitenskapelige Rekke,* **11**: 1–17.

EMERSON, D. N., 1967. Some aspects of free amino acids metabolism in developing encysted embryos of *Artemia salina,* the brine shrimp. *Comp. Biochem. Physiol.,* **20**: 245–261.

ENGEL, D. W., AND J. W. ANGELOVIC, 1968. The influence of salinity and temperature upon the respiration of brine shrimp nauplii. *Comp. Biochem. Physiol.,* **26**: 749–752.

GEER, B. W., 1963. A ribonucleic acid-protein relationship in *Drosophila* nutrition. *J. Exp. Zool.,* **154**: 353–364.

GILCHRIST, B. M., 1960. Growth and form of the brine shrimp *Artemia salina* (L.). *Proc. Zool. Soc. London,* **134**: 221–235.

GILLES, R., 1970. Intermediary metabolism and energy production in some invertebrates. *Arch. Inter. Physiol. Biochem.,* **78**: 313–326.

HERNANDORENA, A., 1970. Obtentions de morphogenèses appendiculaires abortives et sur-numéraires chez *Artemia salina* (L.) (Crustacé branchiopode) par carences alimentaires de base pyrimidique et de nucléotide purique. *C. R. Hebd. Séanc. Acad. Sci. Paris, série D,* **271**: 1406–1410.

HERNANDORENA, A., 1972a. Signification morphogénétique du besoin alimentaire en acides nucléiques chez *Artemia salina* (L.). I-Besoin en thymidine. *Arch. Zool. Exp. Gen.,* **113**: 425–432.

HERNANDORENA, A., 1972b. Signification morphogénétique du besoin alimentaire en acides nucléiques chez *Artemia salina* (L.). II—Besoin en dérivés puriques. *Arch. Zool. Exp. Gen.,* **113**: 489–498.

HERNANDORENA, A., 1972c. Evidence for xanthine dehydrogenase action on lipid metabolism in *Artemia salina* (L.). *Comp. Biochem. Physiol.,* **42A**: 939–944.

HINTON, T., J. F. ELLIS AND D. T. NOYES, 1951. An adenine requirement in a strain of *Drosophila. Proc. Nat. Acad. Sci. U. S. A.,* **37**: 293–299.

HUGGINS, A. K., AND K. A. MUNDAY, 1968. Crustacean metabolism. *Adv. Comp. Physiol. Biochem.,* **3**: 271–378.

HUGGINS, A. K., 1969. The metabolism of 2-^{14}C sodium acetate by the brine shrimp *Artemia salina* and the effect of alterations in salinity. *Comp. Biochem. Physiol.,* **29**: 439–445.

KOBAYASHI, K., Y. SAITA AND T. TOMIYAMA, 1972. Incorporation of $^{32}PO_4$ directly taken up into acid-soluble phosphates of *Artemia salina. Mar. Biol. Germ.,* **12**: 295–299.

KON, R. T., AND R. E. MONROE, 1971. Utilization of dietary amino acids in lipid synthesis by aseptically reared *Musca domestica. Ann. Entomol. Soc. Amer., U. S. A.,* **64**: 247–250.

MAETZ, J., AND R. SKADHAUGE, 1968. Drinking rates and gill ionic turnover in relation to external salinities in the eel. *Nature,* **217**: 371–373.

MCWHINNIE, M. A., AND A. J. CORKILL, 1964. The hexosemonophosphate pathway and its variation in the intermolt cycle in crayfish. *Comp. Biochem. Physiol.,* **12**: 81–93.

McWhinnie, M. A., and R. J. Kirchenberg, 1962. Crayfish hepatopancreas metabolism and
    the intermoult cycle. *Comp. Biochem. Physiol.,* **6** : 117–128.

Packard, T. T., and P. B. Taylor, 1968. The relationship between succinate dehydrogenase
    activity and oxygen consumption in the brine shrimp *Artemia salina.* *Limnol.
    Oceanogr.,* **13** : 552–555.

Provasoli, L., and A. d'Agostino, 1969. Development of artificial media for *Artemia salina.
    Biol. Bull.,* **136** : 434–453.

Reeve, M. R., 1963. The filter feeding of *Artemia.* I—In pure cultures of plant cells. *J. Exp.
    Biol.,* **40** : 195–205.

Renaud, L., 1949. Le cycle des réserves organiques chez les crustacés décapodes. *Ann. Inst.
    Océanogr.* (Paris), **24** : 259–357.

Sang, J. H., 1959. Circumstances affecting the nutritional requirements of *Drosophila mel-
    anogaster. Ann. New York Acad. Sci.,* **77** : 352–365.

Thayer, D. W., 1972. Effect of dietary amino acid on the amino acid pool of *Aedes aegypti.
    J. Insect. Physiol.,* **18** : 521–526.

Vonk, H. J., 1960. Digestion and metabolism. Pages 291–316 in T. H. Waterman, Ed., *The
    Physiology of Crustacea.* Vol. 1. New York, Academic Press.